Effect of Intrathecal Non-NMDA EAA Receptor Antagonist LY293558 in Rats

A New Class of Drugs for Spinal Anesthesia

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Background: Excitatory amino acid receptors are important for both sensory and motor function in the spinal cord. We studied the effects of intrathecal LY293558, a competitive non-N-methyl-D-aspartate excitatory amino acid receptor antagonist, on motor and sensory function in rats to determine whether drugs blocking these receptors could potentially be used as alternative agents to local anesthetics for spinal anesthesia.

Methods: Rats were tested before and 15–240 min after intrathecal injection of 5 nmol (in 10 μl) LY293558. Sensory function was tested at the hind paw using withdrawal response to pin prick and withdrawal to pinch with sharp forceps. Motor performance (ambulation, placing reflex, and Rotorod time), blood pressure, and heart rate were also evaluated. Some tests were repeated the next day. Responses after LY293558 were compared to injection of 40 μl bupivacaine, 0.75%. Pin-prick responses at the forepaw, chest, abdomen, hind leg, and hind paw were also examined after intrathecal LY293558.

Results: Intrathecal LY293558 blocked both sensory and motor responses through 180 min; complete recovery was present the following day. No change in blood pressure or heart rate occurred. The effects of LY293558 were more pronounced and sustained than those of bupivacaine. Segmental blockade of the response to pin prick was present after LY293558.

Conclusion: Drugs like LY293558 that block α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)/kainate receptors may be an alternative to local anesthetics for spinal anesthesia in humans.

SPINAL anesthesia using local anesthetics is associated with acute side effects, including hypotension and urinary retention, persistent sequelae like transient neurologic symptoms, and rarely permanent deficits like cauda equina syndrome.1,2 Current research in spinal anesthesia has focused on the incidence of transient neurologic symptoms and the dose and particular local anesthetic used, the effect of additives like epinephrine, and associated factors like patient position.3,4 There has been no progress in advancing new drugs for spinal anesthesia.

One alternative to conduction block for spinal anesthesia is blockade of synaptic transmission in the spinal cord. This could be accomplished by activation of inhibitory receptors or by antagonism of excitatory receptors. Glutamate is the major excitatory central nervous system neurotransmitter. Glutamate activates ionotropic excitatory amino acid (EAA) receptors that are highly prevalent in the nervous system and transmit information through both N-methyl-D-aspartate (NMDA) as well as non-NMDA EAA receptors.5 Ketamine, a drug used clinically in anesthesia, antagonizes NMDA receptors. Thus far, a clinical use for non-NMDA receptor antagonists, blocking α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate (KA) receptors, has not been discovered.6 In experimental animals, spinally administered non-NMDA receptor antagonists have been shown to inhibit nociception and produce motor dysfunction.7,8 This effect likely results from antagonism of non-NMDA receptors on dorsal horn sensory neurons as well as motor neurons in the ventral horn of the spinal cord. Through drug development, more potent and specific non-NMDA receptor antagonists like LY2935589 have been discovered. The purpose of this study was to determine whether intrathecal injection of LY293558 could produce spinal anesthesia in rats. A comparison to 0.75% bupivacaine is made.

Methods

The Animal Care and Use Committee at the University of Iowa (Iowa City, Iowa) approved these experiments, and the animals were treated in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals. Male Sprague-Dawley rats (n = 50), 250–350 g (Harlan, Indianapolis, IN), were housed in pairs before surgery and were housed individually afterward. Food and water were available ad libitum. At the end of protocol, rats were euthanized using a phenytoin–phenobarbital mixture.

Surgery

Rats were anesthetized with halothane. As described previously,10 the lumbar region was shaved, prepared with povidone–iodine, made kyphotic, and incised 2 to 3 cm longitudinally in the midline at the level of the iliac crests. The space between the fifth and sixth lumbar vertebrae was punctured with a 23-gauge hypodermic needle, and a 32-gauge PU catheter (length, 10 cm; OD, 0.0107 in; ID, 0.005 in; Micor, Allison Park, PA), rein-
forced with a Teflon-coated stainless steel stylet (0.003 in; Micor), was advanced through the needle cranially. The needle and the stylet were removed, and the catheter was withdrawn so that 6.5–7 cm extended outside of the lumbar musculature. The catheter was fixed to the fascia, sutured, inserted into an 8-cm length of PE-10 tubing (Becton Dickinson, Sparks, MD), and secured. The catheter was tunneled under the skin to the cervical region, flushed with saline, and sealed with cautery. The dead space of the catheter was 4.5–5 μl. The skin was closed, and the rat was allowed to recover for 2 to 3 days.

For blood pressure measurement, the paratracheal region was shaved, prepared with povidone–iodine, and incised 1.5–2 cm longitudinally lateral to the midline. The carotid artery was isolated and cannulated with PE-50 tubing that had been stretched by exposing the distal end to heat. The secured catheter was tunneled subcutaneously to the posterior neck region. The skin was closed with 4-0 silk, and the rat recovered for 2 to 3 days before experiments began.

Testing Procedures

Motor, sensory, and hemodynamic measures were recorded on separate days. Measurements were made before drug or vehicle injection (pre) and 15, 30, 60, 90, 120, 180, and 240 min after injection. Some tests were repeated at 24 h. Some rats were used for more than one (but not more than two) experiment(s). If a rat was used more than once, there were 2 days between experiments.

Blood Pressure and Heart Rate

Mean arterial pressure and heart rate were measured at the times shown in figure 1. The carotid artery catheter was attached to a pressure transducer and relayed to a polygraph and preamplifier (Grass Instruments, Quincy, MA; model 7P511J). Heart rate was intermittently measured by counting the arterial pulse wave for 30 s.

Sensory Testing

First, the withdrawal response to pin prick was tested on each hind paw. A 5-mm-long tip of a safety pin attached to a von Frey filament (520 mN) was applied once to the plantar aspect of each hind paw. If either withdrawal or a response (vocalization or attempt to withdrawal) to pin prick occurred, a 1 was recorded. If no withdrawal or response (vocalization or attempt to withdrawal) was observed, a 0 was recorded. The sum of the scores (0, 1, or 2) for each rat was recorded. If no response was elicited by pin prick, serrated dissecting forceps were used to pinch the skin of the plantar region for 2 to 3 s. Again, withdrawal or response (vocalization or attempt to withdrawal) was considered a positive test result (score = 1), and the absence of both was considered a negative test result (score = 0). In preliminary studies, after drug administration, some rats did not respond to pin prick but responded to pinch, but all rats that did not respond to pinch also failed to respond to pin prick. Because the response to pinch was quite vigorous and rats were subjected to repeated tests in a single day, only rats unresponsive to pin prick were tested for pinch. If pin prick evoked a response, pinch was designated as positive also. The sum of the scores after pinch (0, 1, or 2) was recorded.

In a separate group of rats (n = 5), the response to pin prick was tested once on the left hind paw, the left hind limb, the lower abdomen, the thorax, and the left forepaw in rats injected with intrathecal LY293558. Withdrawal, muscle contraction, or attempted escape to pin prick was a positive response, whereas their absence was negative.

Withdrawal to pin prick was also tested before and after infiltration (50 nmol in 100 μl) of LY293558 into the left hind paw (n = 5). Responses were recorded...
before injection of LY293558 and at 15, 30, and 60 min after injection.

Motor Function

Rats were trained over a 2-day period. Training began by placing the rat on a Rotorod (Stoelting, Wood Dale, IL) that was fixed for 3 min. Then, three series of trials separated by 2 to 3 h were performed. During each trial, the rat was placed on the Rotorod, accelerating for the first 20 s and then at a constant rate of approximately 5 rpm, until a 180-s cutoff time. If the rat fell at any time during the initial trial, the trial was repeated. Each rat was allowed three attempts per trial. On the second day, the same procedure was repeated, though acceleration was sustained for 120 s to approximately 12 rpm, with a 150-s cutoff time. On day 3, the test day, rats were again placed on the Rotorod and accelerated for 120 s to 12 rpm with a cutoff time of 150 s. Each rat was permitted four attempts at 15-min intervals. Those successful in two of the four attempts continued in the protocol, and the average of the four attempts was considered the baseline (pre). After a 2-h period, the test drug was administered, and rats were examined once on the Rotorod at 30, 60, 90, 120, 180, and 240 min. They were again tested the following day (24 h).

Ambulation (walking) was observed for approximately 1 min (2 = normal; 1 = limping; 0 = paralyzed) once every test period after the Rotorod test. Then, the placing reflex was tested. Rats were placed on a table, and the dorsum of either hind paw was drawn across the edge of the table\textsuperscript{11}; this elicits a lifting of the paw onto the surface of the table (2 = normal; 1 = delay of 1 to 2 s; 0 = more than 2 s). This test was performed three times for each hind limb after each ambulation test. The sum of the three trials on both paws was recorded (0–12).

The person evaluating the responses was blinded to the drug administered. For responses to pin prick after hind paw infiltration, there was no blinding. Also, we did not blind for responses to pin prick at the forepaw, thorax, abdomen, hind limb, and hind paw after intrathecal LY293558.

Drugs

The dose and volume of drugs injected intrathecally were determined from the dose required to produce hind limb sensory and motor loss in preliminary experiments. Bupivacaine HCl (0.75% in 8.25% dextrose for spinal injection) was purchased from Abbott Laboratories (Chicago, IL). The intrathecal injection volume was 40 \( \mu l \). Five nanomoles of LY293558, a gift from Eli Lilly Corporation (Indianapolis, IN), was dissolved in 10% dextrose and water. The injection volume was 10 \( \mu l \). All intrathecal drug injections were followed by a flush of 10 \( \mu l \) preservative-free saline.

Statistical Analyses

The results are presented as median for ordinal data and mean ± SD for continuous data. Ordinal data were compared using nonparametric analyses. The Friedman test for within-group and the Wilcoxon–Mann–Whitney rank sum test for between-group comparisons were used. Multiple comparisons versus baseline (pre) following the Friedman test were made using a Dunnett test.\textsuperscript{12} Because baseline motor and sensory tests were already at cutoff and could only decrease after drug administration, a one-tailed test was used. \( P < 0.05 \) was considered significant.

Results

Fifteen minutes after intrathecal administration of bupivacaine (fig. 1), mean arterial pressure decreased and heart rate increased (\( P < 0.05 \) vs. pre). These changes were short-lived, resolving by 30 min. Intrathecal LY293558 did not change blood pressure or heart rate. Ambulation, the placing reflex, and Rotorod performance (table 1) were decreased for 30 min after intrathecal bupivacaine administration (\( P < 0.05 \) vs. pre). Withdrawal to pin prick was reduced 15 min after bupivacaine injection (\( P < 0.05 \) vs. pre) but was not eliminated in all tests (fig. 2). All rats had recovered 60 min later. The response to pinch was reduced 15 min after bupivacaine injection (\( P < 0.05 \) vs. pre) with full recovery at 30 min and thereafter.

Table 1. Effect of IT LY293558 and Bupivacaine on Motor Function

<table>
<thead>
<tr>
<th>Time</th>
<th>Cumulative Placing Score</th>
<th>Ambulation Score</th>
<th>Rotorod Time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 nmol LY293558</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>12 (12–12)</td>
<td>2 (2–2)</td>
<td>144 ± 10</td>
</tr>
<tr>
<td>15 min</td>
<td>0 (0–0)*†</td>
<td>0 (0–0)*</td>
<td>3 ± 4*†</td>
</tr>
<tr>
<td>30 min</td>
<td>0 (0–0)*†</td>
<td>0 (0–0)*†</td>
<td>3 ± 0*†</td>
</tr>
<tr>
<td>60 min</td>
<td>0 (0–0)*†</td>
<td>0 (0–0)*†</td>
<td>7 ± 12*†</td>
</tr>
<tr>
<td>90 min</td>
<td>0 (0–0)*†</td>
<td>0 (0–1)*†</td>
<td>7 ± 14*†</td>
</tr>
<tr>
<td>120 min</td>
<td>1.5 (0–11)*†</td>
<td>1 (1–2)*†</td>
<td>11 ± 13*†</td>
</tr>
<tr>
<td>180 min</td>
<td>11 (3–12)*†</td>
<td>2 (1–2)*†</td>
<td>68 ± 29*†</td>
</tr>
<tr>
<td>240 min</td>
<td>12 (8–12)*†</td>
<td>2 (2–2)</td>
<td>115 ± 42*</td>
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<td>24 h</td>
<td>12 (12–12)</td>
<td>2 (2–2)</td>
<td>149 ± 5</td>
</tr>
<tr>
<td>0.75% Bupivacaine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>12 (12–12)</td>
<td>2 (2–2)</td>
<td>146 ± 8</td>
</tr>
<tr>
<td>15 min</td>
<td>0 (0–0)*</td>
<td>0 (0–0)*</td>
<td>5 ± 6*</td>
</tr>
<tr>
<td>30 min</td>
<td>6 (0–12)*†</td>
<td>1 (0–2)*</td>
<td>49 ± 42*†</td>
</tr>
<tr>
<td>60 min</td>
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</tr>
<tr>
<td>24 h</td>
<td>12 (12–12)</td>
<td>2 (2–2)</td>
<td>150 ± 0</td>
</tr>
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</table>

Tests for motor impairment before drug injection (Pre) and 15–240 min and 24 h after intrathecal administration of LY293558 (\( n = 8 \)) or bupivacaine (\( n = 8 \)). Placing reflex and ambulation are expressed as median and range. Rotorod time is expressed as mean ± SD.

\* \( P < 0.05 \) versus Pre by Friedman and Dunnett test. † \( P < 0.05 \) versus bupivacaine by Mann–Whitney rank sum test.

Anesthesiology, V 97, No 1, Jul 2002
LY293558 significantly impaired (table 1) ambulation from 15 through 90 min ($P < 0.05$ vs. pre). All rats had recovered by 4 h. The placing reflex was completely eliminated in all rats through 90 min and impaired through 3 h ($P < 0.05$ vs. pre). Rotorod performance was decreased through 4 h after LY293558 injection ($P < 0.05$ vs. pre); full recovery was evident the next day.

After intrathecal LY293558, rats failed to respond to pin prick (fig. 2) from 15 min through 2 h ($P < 0.05$ vs. pre). Similarly, all rats failed to respond to pinch from 15 through 90 min ($P < 0.05$ vs. pre). These nociceptive responses recovered fully the next day. LY293558 produced greater motor deficits than bupivacaine ($P < 0.05$) from 30 min through 3 h in all tests of motor function. LY293558 produced greater inhibition of pin prick and pinch ($P < 0.05$) than bupivacaine through 3 and 4 h, respectively. No rats vocalized or appeared agitated in response to pin prick or pinch after either drug.

Pin-prick testing at the hind paw, leg, abdomen, chest, and forepaw demonstrated that intrathecal LY293558 blocked sensation below the thorax in all rats through 90 min, and in the hind limb through 120 min (fig. 3). There was evidence of sensory block of the forelimb in only one test at 15 min.

When LY293558 was injected into the hind paw, there was no change in response from baseline. All five rats withdrew to pin prick at 15, 30, and 60 min (data not shown).

**Discussion**

Intrathecally administered LY293558 produced reversible, sustained sensory and motor blockade of the hind limbs in rats. Bupivacaine decreased sensory and motor responses, but the effect of LY293558 was more pronounced and prolonged. These data indicate that non-NMDA EAA receptor antagonists like LY293558 may be useful for producing spinal anesthesia in humans.

Clinically, intrathecally administered local anesthetics produce spinal anesthesia by interfering with axonal conduction of the sensory and motor nerve roots. A contribution by blockade of synaptic transmission and/or conduction within the spinal cord by local anesthetics may also occur. Conduction blockade does not likely contribute to the sensory and motor deficits caused by intrathecal LY293558 because infiltration with 50 nmol in 100 µl LY293558 did not affect the withdrawal response to pin prick. We have observed that plantar infiltration with 100 µl bupivacaine, 0.5%, produces anesthesia to pin prick for 45 min (unpublished observation). Therefore, intrathecally administered LY293558 presumably produces segmental sensory and motor blockade.
spinal anesthesia by blockade of excitatory synaptic transmission of both motor and sensory pathways in the spinal cord.

LY293558 is a competitive AMPA/KA receptor antagonist that has high affinity for AMPA receptors.13 The non-NMDA receptor system is further refined by experiments using recombinant receptor subunits that form homomeric and heteromeric multimers in vivo with relative selectivity for AMPA or KA. Four genes encode AMPA receptor subunits, and five encode the KA receptor subunits.5,6 Glutamate receptor (GluR) subunits 1, 2, 3, and 4 are selective but not specific for AMPA; the GluRs 5, 6, and 7 (low-affinity KA) and the KAs 1 and 2 (high-affinity KA) assemble into receptors of the KA-preferring type. The rank order of potency for displacing AMPA binding by LY293558 is GluR2 > GluR1 > GluR3 > GluR4; the drug has high affinity (< 10 μM) for GluR 1 and 2. LY293558 also displaces KA binding from GluR5 with similar affinity but weakly antagonizes agonist binding to GluRs 6 and 7 and KA2 receptors.13,14 Blockade at KA receptors, particularly GluR5, may contribute to the anesthetic effect. In vivo, LY293558 does not affect NMDA-induced excitation of dorsal horn neurons, but in vitro, it does bind to the NMDA receptor. One limitation of our study is that we cannot be assured that the drug is only affecting the AMPA/KA receptors after administration of 5 nmol.

Rats did not vocalize or appear agitated in response to pin prick or pinch after either drug. This indicates that the loss of response to noxious stimuli was not purely motor blockade. In a preliminary report, we examined the analgesic and antihyperalgesic effects of intrathecally administered LY293558, 0.2–2 nmol, in rats after plantar incision.15 In these studies, inhibition of pain behaviors outlasted the effects on motor function. In the present study, 180 min after administration of 5 nmol of LY293558, rats recovered enough motor function to run on the Rotorod for an average of 68 s (table 1), but only 4 of 16 responses to pin prick and 4 of 16 responses to pinch were evoked. Inhibition of pain responses was not due purely to motor blockade.

Glutamate receptors are present throughout the dorsal and ventral horns of the spinal cord.16–18 Receptor subtypes GluR 1, 2, 3, 5, and 7 are present in the dorsal horn and on primary afferent terminals (GluR5).15 Given this distribution, blockade of these receptors should inhibit transmission of touch and position sense as well as responses to noxious stimuli. In support of this, antagonism of spinal non-NMDA receptors eliminates responses of dorsal horn neurons to touch and pinch.19,20 In the ventral horn, GluRs 2–7 are concentrated within lamina IX, the location of the ventral motor neurons.17,21 Non-NMDA receptor antagonists inhibit activity in motor neurons; thus, the hind limb paresis after intrathecal injection is expected.

Despite the potential for toxicity, attempts have been made to use ketamine, an NMDA receptor antagonist, for spinal anesthesia in humans.23–25 Trials using intrathecal ketamine showed inadequate anesthesia and psychomimetic disturbances and led to the conclusion that ketamine was unsuitable as a single agent for spinal anesthesia.25 Also, adverse effects including sedation, dizziness, nystagmus, and nausea and vomiting suggesting supraspinal spread limited dosing.26 We attempted to administer high doses of NMDA receptor antagonists intrathecally to rats and observed motor impairment without sensory block or supraspinal side effects.8 When local anesthetics are used for spinal anesthesia, they produce hypotension by blocking axonal conduction in preganglionic fibers originating from the intermediolateral cell column in the thoracolumbar spinal cord. Hypotension and the baroreflex-mediated tachycardia after intrathecal bupivacaine were transient in rats. It has been shown that the sympathetic vasomotor center in

![Fig. 3. (A) Percent responses to pin prick after intrathecal injection of LY293558 (n = 5). ▲ Represents the forepaw, □ the chest, ■ the abdomen, ○ the leg, and ● the hind paw. (B) Schematic figure of a rat, depicting the locations for responses to pin prick.](image-url)
the brainstem activates monosynaptically the intermediolateral neurons via the non-NMDA (AMPA) receptor. 

Thus, LY293558 would be expected to decrease sympathetic outflow and blood pressure. It was surprising that LY293558 did not produce hypotension. This lack of hypotension by LY293558 could permit spinal anesthesia in patients with suboptimal hemodynamic status. Because the catheter tip is usually at the midlumbar level, it is possible the blockade was too caudal to affect thoracolumbar sympathetic outflow. Alternatively, the physicochemical properties of the drug may render the intermediolateral cell column less susceptible to blockade by intrathecal LY293558 than sensory and motor neurons in the rat.

There are limitations to translating to human spinal anesthesia using a small rodent model. The length of the spinal axons exposed to drugs is much shorter; this probably reduces the efficacy and duration of intrathecal local anesthetics. In rats, sensory and motor functions were inhibited by bupivacaine, but not to the degree observed in humans undergoing spinal anesthesia. The horizontal posture of the rat may not allow for a sufficient caudal concentration of bupivacaine for spinal anesthesia in rats. The dose of bupivacaine was increased to produce the greatest inhibition of sensory and motor function in the hind limbs, but this dose could not be greater due to cerebral spread. Since we used hyperbaric solutions for both drugs, the segmental effect of LY293558 should be replicable using similar drugs in humans.

Further study using this class of drugs may advance anesthesia. Drugs with greater specificity for a particular GluR subtype may be useful for particular clinical scenarios demanding a relatively greater sensory versus motor blockade. Shorter-acting non-NMDA receptor antagonists may be synthesized. Drugs such as LY293558 may be used as sole anesthetics or as adjuvant agents. It must be recognized that only a limited number of human studies using parenterally administered LY293558 have been completed, and dosing is limited by side effects. Any consideration of clinical trials must be preceded by safety studies in animals using suitable formulations for spinal administration, and these are not currently available.

References